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Intensity-dependent contribution of neuromuscular fatigue after constant-load cycling

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Running head: Fatigue after constant-load cycling

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Purpose. We tested the hypothesis that central and peripheral fatigue after constant-load cycling exercise would vary with exercise intensity and duration. **Methods.** Twelve, well-trained male cyclists ($\dot{V}O_{2\max}$, 4.49 ± 0.35 L·min⁻¹) completed three constant-load cycling trials to the limit of tolerance in a randomized, crossover design. Exercise intensities were set according to the respiratory responses to a preliminary ramp test to elicit cardiorespiratory and metabolic responses consistent with exercise in the severe and heavy exercise domains; 1) at power at $\dot{V}O_{2\max}$ (S+, 379 ± 31 W), 2) at 60% of the difference between gas exchange threshold and $\dot{V}O_{2\max}$ (S-, 305 ± 23 W) and 3) at the respiratory compensation point (RCP, 254 ± 26 W). Pre- and post-exercise twitch responses from the quadriceps to electrical stimulation of the femoral nerve and magnetic stimulation of the motor cortex were recorded to assess neuromuscular and corticospinal function, respectively. **Results.** Exercise time was 3.14 ± 0.59 min, 11.11 ± 1.86 min and 42.14 ± 9.09 min for S+, S- and RCP respectively. All trials resulted in similar reductions in maximum voluntary force ($P=0.61$). However, the degree of peripheral fatigue varied in an intensity-dependent manner, with greater reductions in potentiated twitch force after S+ ($-33 \pm 9\%$) compared to both S- ($-16 \pm 9\%$, $P<0.001$) and RCP trials ($-11 \pm 9\%$, $P<0.001$) and greater after S- compared to RCP ($P<0.05$). For central fatigue this trend was reversed, with smaller reductions in voluntary activation after S+ compared to RCP ($-2.7 \pm 2.2\%$ vs. $-9.0 \pm 4.7\%$, $P<0.01$). **Conclusion.** These data suggest the magnitude of peripheral and central fatigue after locomotor cycling exercise is exacerbated with exercise intensity and duration, respectively.

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Key words: Central, locomotor exercise, muscle, peripheral, voluntary activation, transcranial magnetic stimulation.

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Introduction

Fatigue is an exercise-induced impairment in the ability to produce muscular force (19) in the presence of an increased perception of effort (16). The neuromuscular mechanisms contributing to fatigue can be broadly attributed to processes along the motor pathway that are deemed central or peripheral in origin. For high-intensity locomotor cycling exercise, a consistent magnitude of peripheral fatigue (~35% reduction in potentiated quadriceps twitch force) has been documented at exercise termination under various experimental conditions (2-6). This observation has been proposed to represent a centrally mediated “individual critical threshold” regulated by inhibitory afferent feedback to protect against excessive disruption to homeostasis (1). Although the consistency of end-exercise peripheral fatigue in these studies is striking, whether this plays a determining role in exercise tolerance is controversial, and recent evidence suggests any individual critical threshold is likely to be task-dependent. For example, subsequent work from the same group has demonstrated greater end-exercise peripheral fatigue after dynamic single compared to double-limb exercise (32, 33), and Johnson *et al.* (25) recently reported end-exercise peripheral fatigue after high-intensity cycling is attenuated, central fatigue is exacerbated, and exercise tolerance is reduced if exercise is preceded by high-intensity arm-cranking.

For self-paced cycling time-trial exercise, the magnitude of end-exercise peripheral fatigue also varies with the intensity and duration of the task. We recently demonstrated greater peripheral fatigue after short-duration, high-intensity 4 km self-paced time-trials in comparison to longer, lower-intensity 20 and 40 km time-trials, where central fatigue was exacerbated (38). It was hypothesized that the task-dependent nature of the fatigue observed after self-paced cycling time-trials could be explained by differences in the exercise intensity domain in which the trials were predominantly completed. The exercise intensity domain

concept describes the distinct physiological responses that occur during exercise above and below an individual's maximum sustainable intensity, or critical power (10, 26, 31). The shorter, high-intensity 4 km trial, where peripheral fatigue was higher, was characterized by non-linear physiological responses consistent with exercise in the severe-intensity domain (38). In contrast the longer time-trials, where peripheral fatigue was lower but central fatigue was exacerbated, were characterized by steady-state physiological responses for the majority of the trial, consistent with sustainable exercise below critical power in the heavy exercise intensity domain (38). The distinct physiological responses associated with non-steady state, severe-intensity exercise compared to steady-state, heavy-intensity exercise might therefore be associated with a distinct profile of neuromuscular fatigue. However the varying nature of self-selected power output in these trials meant participants were traversing between exercise intensity domains, particularly at the start and end of the trial, limiting the validity of this conclusion.

These limitations notwithstanding, a similar intensity and duration-dependent pattern of central and peripheral processes to fatigue has been observed for exhaustive, intermittent, single-limb exercise above and below critical torque (10). Interestingly, this study reported that at varying intensities above critical torque, central fatigue was exacerbated in a duration-dependent manner, but the decline in potentiated twitch force was consistent between trials (10). This similar decline in potentiated twitch force suggests that peripheral fatigue is a primary determinant of exercise tolerance during single-limb contractions at intensities above critical torque. Whether this posit can be extended to locomotor exercise above critical power in the severe-intensity domain is not known. Recently, de Souza *et al.* (13) demonstrated no difference in the magnitude of end-exercise fatigue after exercise in the severe-intensity domain of 3 min vs. 10 min duration, but the contribution of central and peripheral processes

was not assessed. Given 1) the accelerated development of fatigue and associated metabolic perturbations at severe exercise intensities (10, 26, 31), 2) the consistency of end-exercise peripheral fatigue after high-intensity cycling exercise (2-6) and 3) the consistency of exercise-induced fatigue after severe-intensity cycling of varying durations (13) it makes the expectation tenable that exercise termination during short duration, high-intensity exercise above critical power in the severe-intensity domain would be concurrent with a consistent magnitude of peripheral fatigue. Conversely, longer duration, steady-state exercise in the heavy domain likely terminates with a smaller magnitude of peripheral fatigue, but a greater contribution from central fatigue mechanisms (10, 38). Accordingly, the aims of this study were to test whether central and peripheral fatigue would differ after short-duration, severe-intensity constant-load locomotor exercise compared to longer-duration, heavy-intensity exercise, and to assess whether exercise at intensities in the severe domain would terminate with a similar degree of peripheral fatigue. We hypothesized that 1) central fatigue would be exacerbated in a duration-dependent manner, 2) that peripheral fatigue would be lower after long-duration constant-load cycling exercise compared to short-duration, severe-intensity cycling exercise, and 3) that exercise in the severe-intensity domain would terminate with a similar degree of peripheral fatigue.

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Methods

120 *Participants*

121 Twelve well-trained male cyclists (mean \pm SD age, 28 ± 8 years; stature, 1.80 ± 0.05 m, body
122 mass, 76 ± 8 kg; maximum oxygen uptake ($\dot{V}O_{2\max}$), 4.49 ± 0.35 L \cdot min $^{-1}$; power at
123 $\dot{V}O_{2\max}$, 399 ± 31 W) gave written informed consent to volunteer. Ethical approval was
124 obtained from the Northumbria University Faculty of Health & Life Sciences Ethics
125 Committee. All participants were in regular cycling training and competition in road and/or
126 time-trial disciplines.

127

128 *Design*

129 Participants visited the lab on four separate occasions to complete a preliminary ramp test,
130 followed by three experimental constant-load cycling trials to the limit of tolerance in a
131 randomized, crossover design. Exercise intensities were set relative to the cardiorespiratory
132 responses measured during the preliminary ramp test. Two of the trials were set at intensities
133 predicted to elicit times to the limit of tolerance of 2-4 min, and 8-15 min, and physiological
134 responses consistent with exercise above critical power in the severe-intensity domain. One
135 trial was set at an intensity predicted to elicit a time to task failure of >30 min, and steady-
136 state physiological responses consistent with sustainable exercise in the heavy-intensity
137 domain. Trials were conducted at the same time of day (± 1 h), separated by a minimum of
138 two and a maximum of seven days. Prior to each experimental trial participants were
139 instructed to refrain from caffeine (for at least 12 h), strenuous exercise (for at least 24 h) and
140 to arrive at the lab two hours post-prandial in a fully rested, hydrated state. Participants
141 completed a 48 h food and activity diary prior to their first experimental trial and were
142 required to replicate their exercise and nutrition as closely as possible before each subsequent
143 trial. Cardiorespiratory, blood lactate concentration and rating of perceived exertion (RPE)

were recorded during each trial and measures of neuromuscular function were assessed pre- and within 2.5 min post-trial.

Procedures

Preliminary visit

Participants attended the laboratory to complete an incremental ramp test to measure $\dot{V}O_{2\max}$ and respiratory thresholds, and habituate to the measurement tools of the study; specifically, electrical stimulation of the femoral nerve and magnetic stimulation of the motor cortex pre- and post-exhaustive cycling exercise. The incremental ramp test was performed on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, Netherlands), and consisted of cycling at 100 W followed by a continuous ramped increase in power of $30 \text{ W} \cdot \text{min}^{-1}$ to the limit of tolerance. The test was terminated when participants cadence reduced by $>20 \text{ rpm}$ from their self-selected cadence for the test. Expired air was analysed breath-by-breath using an online system (Oxycon Pro, Care Fusion, Hoechberg, Germany). Oxygen (O_2) and carbon dioxide (CO_2) concentrations were analysed via a paramagnetic chemical fuel cell and non-dispersive infrared cell respectively. Before each test the analyzers were calibrated using ambient air and a gas of known O_2 (14.00%) and CO_2 (6.00%) concentrations. Ventilatory volumes were inferred from measurement of gas flow using a digital turbine transducer (volume 0 to 10 L, resolution 3 mL, flow 0 to $15 \text{ L} \cdot \text{s}^{-1}$), calibrated prior to each test using a 3 L syringe (Hans Rudolph Inc. Kansas City, USA), and attached to a face mask via a transducer holder (dead space of 30 mL). The gas-exchange threshold (GET) was determined using multiple parallel methods as follows: 1) the first disproportionate increase in carbon dioxide output ($\dot{V}CO_2$) relative to $\dot{V}O_2$, 2) an increase in the ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$) with no increase in the ventilatory equivalent for carbon dioxide ($\dot{V}_E/\dot{V}CO_2$) and 3) an increase in end-tidal O_2 tension with no fall in end-

tidal CO₂ tension (8, 40). Respiratory compensation point (RCP) was determined from plots of minute ventilation (\dot{V}_E) vs $\dot{V}CO_2$ (8). Maximum oxygen uptake was calculated as the highest 30 s mean value attained prior to test termination, and the end test power was recorded as power at $\dot{V}O_{2max}$ (P_{max}).

Experimental trials

Participants completed three constant-load cycling trials to the limit of tolerance. The exercise intensities were determined from the respiratory indices and associated intensities measured during the preliminary ramp test. Two trials were set to elicit non-steady state physiological responses consistent with exercise in the severe-intensity domain with predicted exercise durations of 2-4 min (hereafter referred to as S+) and 8-15 min (hereafter referred to as S-). The third trial was set at a sustainable intensity (hereafter referred to as RCP), with a predicted exercise duration of >30 min. The S+ trial was set at P_{max} , which approximates the upper limit of the severe-intensity domain. The S- trial was set at an exercise intensity equivalent to 60% of the difference between GET and $\dot{V}O_{2max}$, which would also elicit responses consistent with exercise above critical power in the severe domain, but for a greater duration than S+ (20). The RCP trial was set at the power output associated with RCP, as this intensity approximates an upper limit for sustainable exercise (15). When determining constant-load exercise intensities from a ramp protocol, it is recommended the intensity be adjusted to accommodate the mean rise time of $\dot{V}O_2$ during ramp exercise, which approximates two-thirds of the ramp rate (i.e. 20 W; 41). For all trials this recommendation was adhered to, and 20 W was subtracted from the calculated exercise intensity. Each experimental trial was preceded by a 5 min warm up at 150 W before a “step” increase in exercise intensity. Participants remained seated throughout exercise, and the test was terminated when participants could not maintain a cadence within 10 rpm of their self-

selected cadence for the test. Expired air was measured breath-by-breath throughout. Fingertip capillary blood was sampled post-warm-up, at 5 min intervals during constant-load exercise and 3 min post-trial, and immediately analysed for [lactate] using an automated instrument (Biosen C_Line, EFK Diagnostic, Germany). At the same time points during exercise, participants were asked to provide a rating of perceived exertion (RPE) taking into account all sensations of physical stress, effort and fatigue (9).

Neuromuscular function

Measures of neuromuscular function were assessed pre- and post-exercise using electrical stimulation of the femoral nerve and transcranial magnetic stimulation (TMS) of the motor cortex, with evoked force and electromyographic (EMG) responses recorded from the knee extensors of the dominant leg at rest and during voluntary contraction. Following two practice attempts to ensure adequate potentiation, participants completed three isometric maximum voluntary contractions (MVC) of the knee extensors separated by 60 s rest, with femoral nerve stimulation delivered during and ~2 s post to measure voluntary activation (VA) and potentiated quadriceps twitch force ($Q_{tw,pot}$). Subsequently, TMS was delivered during brief (3-5 s) contractions at 100%, 75% and 50% MVC, separated by 5 s rest, for determination of corticospinal voluntary activation. This procedure was repeated 3 times with 30 s rest between each set. Finally TMS ($\times 8$ stimulations) and electrical nerve stimulation ($\times 5$ stimulations) were delivered during submaximal contraction at 20% MVC for determination of corticospinal excitability. Immediately post-exercise these measures were repeated. In accordance with other similar investigations of exercise-induced fatigue of the knee extensors, the assessment of voluntary activation measured with motor nerve and motor cortical stimulation was completed within 2.5 min of exercise cessation (20, 32, 34, 38). The rapid nature of this procedure is necessary to capture the extent of fatigue elicited by the

exercise before it dissipates (18) and the duration (2 to 2.5 min) was consistent between trials. Further details on these procedures are presented in subsequent sections.

Force and EMG recordings

During stimulations participants sat upright in a custom-built chair with hips and knees at 90° of flexion. A calibrated load cell (MuscleLab force sensor 300, Ergotest technology, Norway) was attached via a non-compliant cuff positioned on the participant's right leg, superior to the malleoli, to measure knee extensor force (N). Surface Ag/AgCl electrodes (Kendall H87PG/F, Covidien, Mansfield, MA, USA) were placed 2 cm apart over the rectus femoris (RF), vastus lateralis (VL) and biceps femoris (BF) to record the compound muscle action potential (M-wave) elicited by electrical stimulation of the femoral nerve, the motor evoked potential (MEP) elicited by TMS and root-mean-square amplitude during isometric and dynamic (cycling) exercise. The position of the electrodes was consistent with SENIAM (Surface EMG for the Non-Invasive Assessment of Muscles) guidelines, and a reference electrode was placed over the patella. Electrode placement was marked with permanent ink to ensure a consistent placement between trials. Signals were amplified; gain $\times 1000$ for EMG and $\times 300$ for force (CED 1902, Cambridge Electronic Design, UK), band-pass filtered (EMG only: 20-2000 Hz), digitised (4 kHz; CED 1401, Cambridge Electronic Design, UK) and analysed off line (Spike2 v7.12, Cambridge Electronic Design, UK).

Motor nerve stimulation

Single electrical stimuli (200 μ s duration) were applied to the right femoral nerve using a constant-current stimulator (DS7AH Digitimer Ltd., Welwyn Garden City, UK) via adhesive surface electrodes (CF3200, Nidd Valley Medical Ltd., Harrogate, UK) at rest and during voluntary contraction. The cathode was positioned over the nerve in the femoral triangle in

the location that elicited the maximum quadriceps twitch amplitude (Q_{tw}) and M-wave (M_{max}) at rest. The anode was positioned midway between the greater trochanter and iliac crest. The optimal stimulation intensity was determined as the minimum current that elicited maximum values of Q_{tw} and M_{max} at rest. To ensure a supramaximal stimulus and account for fatigue-dependent changes in axonal excitability, the intensity was increased by 30%, and was not different between trials (211 ± 71 mA, 215 ± 76 mA, 219 ± 74 mA, $P = 0.13$).

Transcranial magnetic stimulation

Single pulse magnetic stimuli (1 ms duration) were delivered to the left motor cortex via a concave double cone coil (110 mm diameter, maximum output 1.4 T) powered by a monopulse magnetic stimulator (Magstim 200, The Magstim Company Ltd., Whitland, UK). The coil position was tilted and held lateral to the vertex over the area relating to Brodmann Area 4 of the primary motor cortex, generating a posterior-anterior intracranial current flow within the left hemisphere. The exact “hot-spot” (marked in semi-permanent ink for reproducible placement between trials) was determined as the coil position that elicited the largest MEP in the rectus femoris during a submaximal (20% MVC) contraction, and a concurrent small MEP in the antagonist muscle. For measures of corticospinal excitability, active motor threshold (AMT) was determined as the stimulator output that elicited a consistent MEP of approximately 0.2 mV in at least 3 of 5 stimulations during 20% MVC (37). The stimulator output was increased by 20% and was not different between trials ($48 \pm 7\%$, $48 \pm 7\%$ and $47 \pm 6\%$ of mean stimulator output, $P = 0.58$). Setting the stimulator intensity relative to AMT in this manner ensures the resulting MEP is not close to saturation on the stimulus-response curve (39) permitting a suitable window for exercise-induced changes. The use of a sub-maximal contraction allows for the monitoring of the status of smaller motoneurons (29). For measurement of corticospinal voluntary activation, the

stimulator output was set to produce the largest superimposed twitch force during a contraction at 50% MVC (37), which averaged 67 ± 17 N, or $12 \pm 5\%$ of MVC. The stimulation intensity, which was not different between trials ($63 \pm 5\%$, $63 \pm 4\%$ and $62 \pm 4\%$ of mean stimulator output, $P = 0.68$), elicited a large MEP in the RF (approximately 70% of M_{\max} during contractions $\geq 50\%$ MVC) indicating the TMS stimulus activated a high proportion of knee extensor motor units, while causing only a small MEP in the antagonist (0.40 mV on average, or $<10\%$ of RF M_{\max} during knee-extensor contractions). Participants were given specific instructions to achieve a plateau in the target force when contracting at varying force levels whilst receiving TMS (22).

Data analysis

Voluntary activation measured via motor nerve stimulation was quantified using the twitch interpolation method: $VA (\%) = (1 - [SIT/Q_{tw,pot}] \times 100)$, where SIT is the amplitude of the superimposed twitch force measured during MVC, and $Q_{tw,pot}$ is the amplitude of the resting potentiated twitch force assessed 2 s post-MVC. For motor cortical stimulation, voluntary activation was assessed by measurement of the superimposed twitch responses to TMS at 100%, 75% and 50% MVC. As corticospinal excitability increases during voluntary contraction, it is necessary to estimate the amplitude of the resting twitch in response to motor cortex stimulation (21, 39). The amplitude of the estimated resting twitch (ERT) was calculated as the y-intercept of the linear regression between the mean amplitude of the superimposed twitches evoked by TMS at 100%, 75% and 50% MVC and voluntary force; regression analyses confirmed the existence of a linear relationship both pre- and post-exercise ($r^2 = 0.90 \pm 0.05$ and 0.86 ± 0.11 , respectively). Voluntary activation (%) measured with TMS was subsequently calculated as $(1 - [SIT/ERT] \times 100)$. The reproducibility and validity of this procedure for the knee extensors has been previously established (21). The

peak-to-peak amplitude and area of the evoked M_{\max} and MEP responses were quantified offline. Corticospinal excitability was determined as the ratio between the MEP and M-wave responses measured during a 20% MVC from eight cortical and five motor nerve stimulations. Contraction strength was adjusted post-trial to equate to 20% of the fatigued MVC force. Electromyography of the vastus lateralis and rectus femoris during the cycling bout was band-pass filtered in both directions between 20-450 Hz using a 4th order zero lag Butterworth filter. The root mean square of the EMG amplitude was measured over ten consecutive pedal revolutions from the middle of minute 4 of the warm-up, and the first and final minutes of cycling exercise, to quantify changes in neuromuscular activation. Onsets and offsets of EMG bursts were determined visually by the same investigator (11, 25). The warm-up and first minute EMG data were normalized to the M_{\max} measured pre-trial, and the final minute EMG data was normalized to the post-trial M_{\max} (average of three electrical stimulations at rest for both).

Reproducibility coefficients

Typical error as a coefficient of variation (CV, %) and intra-class correlation coefficients (ICC, 23) between the pre-trial scores were calculated to quantify reproducibility of neuromuscular function measures. Reproducibility was high for MVC (ICC = 0.96, CV = 4.4%), $Q_{\text{tw,pot}}$ (ICC = 0.90, CV = 4.8%), motor nerve VA (ICC = 0.89, CV = 3.1%), corticospinal VA (ICC = 0.90, CV = 2.6%) and moderate for ERT (ICC = 0.85, CV = 10.0%), M_{\max} (RF; ICC = 0.70, CV = 24.7%, VL; ICC = 0.79, CV = 25.2%) and MEP/ M_{\max} ratio (RF; ICC = 0.74, CV = 13.6%, VL; ICC = 0.79, CV = 26.3%). The reproducibility of the cardiorespiratory responses (\dot{V}_E , $\dot{V}O_2$, $\dot{V}CO_2$, respiratory exchange ratio, RER and HR) was determined from responses to the standardized 5 min warm-up and was considered high for all measures (ICC range = 0.87 to 0.93, CV range = 4.0% to 5.3%).

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Statistical analysis

All analyses were planned *a priori*. For all neuromuscular measures the expected impact of exhaustive exercise on measures of fatigue were assessed using Student's paired-sample t-tests. One-way repeat measures ANOVA analyses of the pre- to post-exercise change scores were used to assess the effect of exercise intensity (S+ vs. S- vs. RCP) on measures of fatigue and neuromuscular function. Differences between trials for exercise time, power output, and cardiorespiratory and metabolic responses were assessed using the same procedure. Trial (S+ vs. S- vs. RCP) by time (warm-up vs. first min vs. final minute) factorial repeat measures ANOVA were used to assess EMG responses; significant main effects were followed up using one-way repeat measures ANOVA. For all ANOVA analyses, pairwise comparisons were made using Tukey's test. Friedman's ANOVA with *post-hoc* Wilcoxon signed-ranks test were employed for non-parametric data (RPE). The assumptions underpinning these statistical procedures were verified and all data were considered normal. Descriptive data are presented as means \pm SD in text, tables and figures. Statistical analysis was conducted using Graphpad (GraphPad Prism 5, Version 5.03, La Jolla, CA, USA). Statistical significance was assumed at $P < 0.05$.

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Results

339 **Exercise responses.** Power output for S+ (379 ± 31 W) S- (305 ± 23 W) and RCP (254 ± 26
340 W) equated to relative intensities of 100% P_{\max} , $76 \pm 3\%$ P_{\max} and $64 \pm 5\%$ of P_{\max} . The
341 resulting exercise times to the limit of tolerance for each trial were 3.14 ± 0.59 min, $11.11 \pm$
342 1.86 min and 42.14 ± 9.09 min for S+, S- and RCP respectively.

343

344 The mean cardiorespiratory (Figure 1) and blood [lactate] (Figure 2, panel A) responses to S+
345 and S- were consistent with exercise above critical power in the severe-intensity domain, and
346 for RCP consistent with exercise in the heavy domain. For S+ exercise, oxygen uptake rose
347 rapidly (Figure 1, panel B), reaching peak values of $98 \pm 8\%$ of maximum, and peak RER
348 and $\dot{V}CO_2$ were higher compared to S- and RCP exercise ($P < 0.05$). For S- exercise,
349 oxygen uptake also rose rapidly, followed by a gradual increase to task failure where peak
350 values averaged $98 \pm 6\%$ of maximum oxygen uptake. For RCP, an oxygen uptake slow
351 component was observed, with peak values averaging $91 \pm 6\%$ of $\dot{V}O_{2\max}$ (Figure 1, panel
352 B). Blood [lactate] post-exercise was highest after S+ (12.3 ± 1.8 mmol·L⁻¹) compared to
353 both S- (10.6 ± 2.1 mmol·L⁻¹, $P < 0.05$) and RCP (5.9 ± 1.8 mmol·L⁻¹, $P < 0.001$) exercise,
354 and higher after S- compared to RCP ($P < 0.001$) (Figure 2, panel A).

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356 Surface EMG data was successfully sampled for all three trials in nine participants. Estimated
357 muscle activation (RMS/ M_{\max}) was different across time ($P < 0.001$), between trials ($P =$
358 0.001) and responded differently to S+, S- and RCP (time \times trial $P = 0.001$, Figure 3). There
359 were no differences observed in muscle activation during the warm-up between trials. During
360 the 1st minute of exercise, EMG RMS/ M_{\max} was higher in S+ compared to RCP ($P = 0.01$),
361 and in the final minute was higher in S+ compared to both S- ($P = 0.04$) and RCP ($P =$
362 0.001). The increase in EMG RMS/ M_{\max} during S+ exercise was higher than RCP between

warm-up and the first minute of exercise ($P = 0.001$), and higher than both S- ($P = 0.04$) and RCP ($P = 0.001$) between the first and final minute of exercise (Figure 3).

For RPE, participants reported higher peak scores for S+ compared to RCP exercise ($P < 0.05$), with no difference between S+ and S-, or between S- and RCP exercise (Figure 2, panel B). When expressed relative to exercise time, the RPE response was similar between trials (Figure 2, panel C).

Neuromuscular responses pre- and post-exercise. One participant exhibited small responses to TMS (MEP:M_{max} ratio in RF $< 60\%$, superimposed twitch force during 50% MVC $< 5\%$ MVC force). Low MEP:M_{max} ratios and a small SIT are indicative of an incomplete activation of the available motoneuron pool by the magnetic stimulus, which could invalidate the measurement of voluntary activation. This participant was subsequently excluded from analysis of data elicited by TMS.

Exercise resulted in a similar level of global fatigue after all trials, as evidenced by substantial reductions in MVC force ($P < 0.05$) that were not different between trials (-111 ± 54 N, -93 ± 48 N and -98 ± 59 N for S+, S- and RCP exercise, respectively, $P = 0.61$) (Figure 4, panel A). The loss in voluntary force was accompanied by significant peripheral and central fatigue in all trials (all $P < 0.05$, Figure 4); however there were differences in the magnitude of central and peripheral fatigue between trials. Peripheral fatigue ($\Delta Q_{tw,pot}$ amplitude) was greater after S+ exercise ($-33 \pm 9\%$) compared to both S- ($-16 \pm 9\%$, $P < 0.001$) and RCP exercise ($-11 \pm 9\%$, $P < 0.001$), and greater after S- compared to RCP exercise ($P < 0.05$) (Figure 4, panel B). For central fatigue this pattern was reversed. For motor nerve estimates of VA, central fatigue was less after S+ ($-2.7 \pm 2.2\%$) compared to

388 RCP ($-9.0 \pm 4.7\%$, $P < 0.01$) but not after S+ compared to S- ($-6.3 \pm 5.5\%$, $P = 0.056$) or
389 after S- compared to RCP ($P = 0.084$) (Figure 4, panel C). For motor cortical estimates of
390 VA no statistical differences were observed after S+ ($-2.7 \pm 3.5\%$) compared to RCP ($-9.2 \pm$
391 8.0% , $P = 0.06$) or S- ($-7.9 \pm 7.4\%$, $P = 0.07$), or after S- compared to RCP ($P = 0.46$)
392 (Figure 4, panel D). Changes in voluntary activation were not accompanied by any changes in
393 MEP or M-wave properties measured during a 20% MVC (Table 1) or during higher force
394 contractions (Table, supplementary digital content 1, neuromuscular function and surface
395 EMG responses to motor nerve and motor cortical stimulation).
396

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Discussion

398 This study shows that the magnitude of central and peripheral fatigue observed after constant-
399 load locomotor cycling varies with exercise intensity and duration. Specifically, the data
400 suggest a task-dependent contribution of peripheral and central fatigue; peripheral fatigue is
401 exacerbated with increases in exercise intensity, whereas central fatigue tends to increase as
402 exercise intensity decreases and duration increases. For non-steady-state exercise in the
403 severe domain, the degree of end-exercise peripheral fatigue was exacerbated at higher
404 intensities, a finding incongruent with the proposal of an individual critical threshold for
405 muscle fatigue after high-intensity locomotor exercise above critical power.

406

407 Contrary to our hypothesis, the degree of end-exercise peripheral fatigue was not consistent
408 between exercise intensities in the severe domain, a finding that does not support the proposal
409 that muscle fatigue after high-intensity locomotor exercise is regulated to an individual
410 critical threshold. The concept of an individual critical threshold, or sensory tolerance limit,
411 was originally proposed by Amann and colleagues (1-7) who completed a series of studies
412 demonstrating that the magnitude of exercise-induced peripheral fatigue was remarkably
413 similar after locomotor exercise under conditions of pre-fatiguing exercise (3), altered arterial
414 oxygen saturation (4) and, perhaps most convincingly, was exacerbated only under conditions
415 of impaired afferent feedback (2, 5). Similar support has also been provided for “all-out”
416 repeat-sprint exercise with and without pre-fatiguing exercise (24). These studies proposed a
417 feedback loop whereby inhibitory input from group III/IV afferents in the active musculature
418 act on the central nervous system to compel a reduction in exercise intensity in order to
419 prevent excessive homeostatic disruption, and restrain the development of muscle fatigue to a
420 consistent limit that would not be exceeded in normal conditions. Although conceptually
421 attractive, our previous work comparing different durations of cycling time-trial exercise

(38), and evidence from the work of others comparing single vs. double leg exercise (32), have demonstrated that any peripheral limit is task-dependent, a point which the authors of this proposal were careful to emphasise (7). Nonetheless, in the current study we hypothesised that an individual, consistent limit for peripheral fatigue might exist for non-steady-state exercise in the severe-intensity domain. This posit was based on the distinct, non-linear responses to severe-intensity exercise (26), the observation of a consistent reduction in potentiated twitch force of ~35% after high-intensity cycling exercise (2, 3, 5, 6), and the finding that peripheral fatigue is similar after single-limb, intermittent, isometric knee extensor contractions at varying intensities above critical torque (10). Contrary to our original hypothesis, the greater reduction in potentiated twitch force observed after the highest intensity trial suggests the degree of peripheral fatigue incurred after exhaustive locomotor exercise above critical power is intensity- and duration-dependent, and not regulated to a critical limit.

An alternative explanation for the differing magnitude of end-exercise peripheral fatigue observed in this, and other studies (2, 5, 24, 32, 33), could simply be related to the force and motoneuron recruitment strategy required to complete the task. In the current study, the force demands of the task and subsequent activation of muscle were highest in the S+ trial, where peripheral fatigue was also highest; the higher peripheral fatigue could simply be explained by the recruitment and subsequent fatigue of a greater portion of the available motoneuron pool. This proposal would also explain a number of previous observations relating to the critical threshold concept. For example, observations of a greater end-exercise peripheral fatigue after single vs. double-limb exercise (32, 33), and after locomotor cycling exercise with afferent blockade (2, 5), could both be explained by a greater activation of muscle, as evidenced by the higher EMG that was concurrent with greater end-exercise peripheral

fatigue in these experimental trials. Additionally, the highest absolute reductions in potentiated twitch force (measured with single electrical stimuli) have been reported after repeat sprint cycling exercise; -51% (24) compared to a range of -33 to -40% after high-intensity time-trial (5, 38) and constant-load cycling exercise (2). Repeat sprint exercise would theoretically require repeated maximum activation of the locomotor musculature, with compromised recovery, and as such the high force demands of the task would elicit a greater recruitment and fatigue of larger, faster motor units, and a consequent higher level of muscle fatigue. In support of this proposal, Decorte *et al.* (14) demonstrated the increase in quadriceps EMG during exhaustive constant-load cycling was significantly correlated with the reduction in quadriceps potentiated twitch force. A limitation to this explanation is an acknowledgement that estimates of muscle activation via surface EMG are subject to a number of valid critiques (17, 27), not least the insensitivity of measures of muscle activation to small differences in exercise intensities. These limitations notwithstanding, the higher EMG that accompanied the higher power output in the S+ trial gives credence to the interpretation that this is indicative of a higher degree of muscle activation. Thus, the proposal that peripheral fatigue after locomotor exercise can be explained by the force and/or motor unit recruitment strategy required for the task remains plausible, and provides a better fit with the available data than a model which emphasises regulation to an individual critical threshold.

While these data question the concept of an individual critical threshold for peripheral fatigue, the proposal that group III/IV muscle afferent feedback acts in an inhibitory manner to contribute to the regulation of exercise intensity remains a logical proposition. Without such feedback regulation is almost certainly compromised, at least for high-intensity locomotor exercise lasting < 10 min (5). Indeed, Amann *et al.* (2, 5) clearly demonstrate that

when such feedback is blocked with administration of an intrathecal opioid analgesic, participants self-select exercise intensities and/or adopt inappropriate recruitment strategies that result in significant additional peripheral fatigue in comparison to a control, with no improvement in performance. These data clearly support the idea that group III/IV afferent feedback is important for the regulation of high-intensity, locomotor exercise. However, the findings of the present study, previous work comparing different durations of self-paced cycling exercise (38), and the observation that prior fatiguing arm exercise can modulate end-exercise peripheral fatigue in high-intensity cycling (25), indicate that peripheral muscle fatigue contributes to, rather than determines, the decision to terminate or modulate locomotor exercise intensity.

In accordance with our hypothesis, longer duration, heavy-intensity exercise terminated with more central fatigue and less peripheral fatigue compared to severe-intensity exercise. This duration-dependent contribution of central fatigue is similar to that previously observed in self-paced (38) and constant-load cycling (14), and single-limb intermittent contractions (10), and indicates that exercise tolerance in longer duration tasks is mediated to a greater extent by mechanisms of central fatigue. Despite differences in the etiology of neuromuscular fatigue, the sensory perception of exertion rose similarly over time independent of exercise duration/intensity (Figure 2, panel C). This is a consistent response to various exercise tasks under varying conditions, which has prompted the development of holistic models that emphasise a mediating role for sensory perception in the etiology of fatigue (28, 30). Interestingly, the highest RPE at exercise termination was reported in the highest intensity trial, where peripheral fatigue was also highest; a finding concurrent with our previous work in self-paced exercise (38). This could support the idea that afferent feedback contributes to the sensory perception of fatigue and the decision to terminate exercise (30), or it could be

argued that the shorter duration of the trial and the lower degree of central fatigue permitted a higher potential motivation for the task and a consequent higher tolerance to a high perception of effort (28). Although a definitive explanation is elusive, it is clear that the decision to terminate exercise was influenced differently depending on the intensity of the trial, and understanding how the mediating inputs to this decision vary across exercise tasks remains an important endeavour to advance our understanding of the factors that limit or modulate exercise performance.

The task-dependent, intensity-domain specificity of peripheral fatigue observed in the present study after constant-load cycling exercise supports our previous work in different durations of self-paced cycling exercise (38), with one caveat. In this previous study we observed a similar degree of peripheral fatigue after 20 and 40 km time-trial exercise (−31% and −29% on average, respectively) despite significant differences in the duration (32 min vs. 66 min) and average intensity (279 W vs. 255 W) of the bouts (38). We hypothesised that this lack of difference could reflect a similarity in the exercise domain in which the trials were completed, predominantly in the heavy domain and below critical power. However, in the present study using the same femoral nerve stimulation methods, and a similar population of well-trained cyclists, the absolute decline in potentiated twitch force after 45 min of constant-load cycling in the heavy exercise intensity domain was only 11%. Based on this new information and the proposal that peripheral fatigue is determined in a task-dependent manner based on the force and/or motoneuron recruitment strategies required for the task, we now consider it likely that the higher, and similar degree of peripheral fatigue observed after 20 and 40 km self-paced exercise was determined primarily by the finishing sprint, which was similar between trials (38). Such a sprint at the end of the trial is common in self-paced exercise and requires higher force and therefore recruitment of higher threshold motor units.

Collectively, this reasoning could explain the greater degree of peripheral fatigue observed after self-paced exercise compared to the relatively modest degree of peripheral fatigue observed in the present study after a similar duration and intensity constant-load bout. These data therefore suggest that the magnitude of peripheral fatigue observed at the end of a self-paced bout of exercise likely reflects the recent contractile history of the muscle, and emphasises the importance and challenge of developing methods to ascertain the time-course development of fatigue *during* as well as post-exercise.

Corticospinal excitability was unchanged in response to locomotor exercise, when measured >2 minutes post-exercise. This response is common in studies employing locomotor exercise where corticospinal excitability is measured pre- and post-exercise (34, 38). However, the lack of change from pre- to post-exercise might not fully reflect modulations in corticospinal excitability that could occur during exercise. Indeed, when responses are elicited during locomotor exercise using a bespoke experimental set-up, there is evidence to suggest corticospinal excitability is reduced (35) and intracortical inhibition is increased (36), which might therefore contribute to the aetiology of fatigue. The lack of change in measures of excitability commonly observed post-exercise might be explained by a rapid recovery, and the lack of specificity of the task in which it is measured. A limitation of the present study is the measurement of corticospinal excitability took place > 2 min post-exercise during isometric contraction, and as such any change in response to exercise might not be elucidated.

The estimate of exercise intensities in the present study was based on the ventilatory and performance responses to a ramp exercise protocol. Two of these intensities (S+, S-) were set to elicit physiological responses consistent with exercise above critical power in the severe-intensity domain, for exercise durations of between 2-4 min and 8-15 min respectively. In

both of these trials, oxygen uptake progressively rose to values that were within 2% of maximum oxygen uptake at exercise termination (Figure 1), and blood [lactate] was not stabilised (Figure 2, panel A), indicative of exercise in the severe domain. The third trial (RCP) was set at the respiratory compensation point, which approximates the critical power (15), and was designed to elicit exercise durations >30 min and physiological responses consistent with sustainable exercise. Though it is acknowledged that the weight of evidence suggests the RCP and critical power are distinct thresholds (12, 31), the exercise duration in RCP exceeded 30 min for all participants, and the cardiorespiratory (Figure 1) and blood [lactate] (Figure 2, panel A) responses were submaximal and stable for the duration of the RCP trial on a group level, indicative of exercise below critical power in the heavy-intensity domain. However, the range in exercise durations (31 to 59 min) suggests a less precise estimate of this intensity, which was likely in the heavy domain for most, but not all, participants. This limitation notwithstanding, the contribution of central and peripheral processes to fatigue after this longer duration bout were distinctive from the shorter, higher-intensity bouts. A more precise estimate of the critical power would allow future research to better assess any differences in the etiology of neuromuscular fatigue at exercise intensities below critical power.

To conclude, the contribution of central and peripheral processes to fatigue after constant-load cycling exercise differs in a task-dependent manner. Central fatigue is exacerbated as exercise duration increases and intensity decreases, whereas peripheral fatigue is greater at higher intensities and shorter durations of exercise. These data suggest that the extent of end-exercise peripheral fatigue is not regulated to an individual critical threshold, but is determined by the force and/or motor unit recruitment strategies required for the task. These

571 data have implications for the concept of a critical limit for peripheral fatigue and our
572 understanding of the etiology of fatigue under different locomotor exercise conditions.

573

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Figure Legends

Figure 1. Minute ventilation (\dot{V}_E , A), respiratory exchange ratio (B), oxygen uptake ($\dot{V}O_2$, C) and carbon dioxide output ($\dot{V}CO_2$, D) during severe- (S+ ○ and S- ▲) and heavy-intensity (RCP ■) constant-load cycling exercise. Values are mean \pm SD (n = 12). The vertical dashed line on the x axis marks the end of the 5 min warm-up and the start of the constant-load trial. The horizontal error bars on the final data point show the variability in time to the limit of tolerance for each trial. Symbols indicate a statistically significant difference ($P < 0.05$) in the peak scores compared to the following: *different from S-, †different from RCP.

Figure 2. Blood lactate (A), rating of perceived exertion (B) and rating of perceived exertion relative to trial duration (C) responses to severe- (S+ ○ and S- ▲) and heavy-intensity (RCP ■) constant-load cycling exercise. The vertical dashed line on the x axis marks the end of the 5 min warm-up and the start of the constant-load trial. The horizontal error bars on the final data point in (A) and (B) show the variability in time to the limit of tolerance for each trial. Values are mean \pm SD (n = 12). Symbols indicate a statistically significant difference ($P < 0.05$) in the peak trial scores compared to the following: *different from S-, †different from RCP.

Figure 3. Muscle activation estimated from electromyography of the vastus lateralis and rectus femoris during a standardized warm-up and the first and final minute of severe- (S+ ○ and S- ▲) and heavy-intensity (RCP ■) constant-load cycling exercise. Values are mean \pm SD (n = 9) Symbols above error bars indicate a statistically significant difference between trials for the measured time point. Symbols on horizontal lines indicate a statistically

significant interaction between time points (all $P < 0.05$). *different from S–, †different from RCP.

Figure 4. Pre- to post-trial percentage change in maximum voluntary contraction (A), potentiated twitch force (B), voluntary activation measured with motor nerve stimulation (C) ($n = 12$) and voluntary activation measured with cortical stimulation (VA_{TMS}) ($n = 11$) (D) after severe- (S+ and S–) and heavy-intensity (RCP) constant-load cycling exercise. Values are mean \pm SD. Symbols indicate a statistically significant difference ($P < 0.05$) compared to the following: *different from S–, †different from RCP.

Tables

Table 1. Motor evoked potentials (MEP), muscle compound action potentials (M-waves) and MEP/M-wave measured in vastus lateralis (VL) and rectus femoris (RF) during a sub-maximal contraction (20% MVC) pre- and post- constant-load cycling exercise. Values are mean \pm SD ($n = 11$).

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Supplemental Digital Content

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Table, Supplementary Digital Content 1. Neuromuscular function and surface EMG

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responses (in *rectus femoris*) to motor nerve (n = 12) and motor cortical (n = 11) stimulation

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during contraction and at rest, pre- and post- severe- (S+ and S-) and heavy-intensity (RCP)

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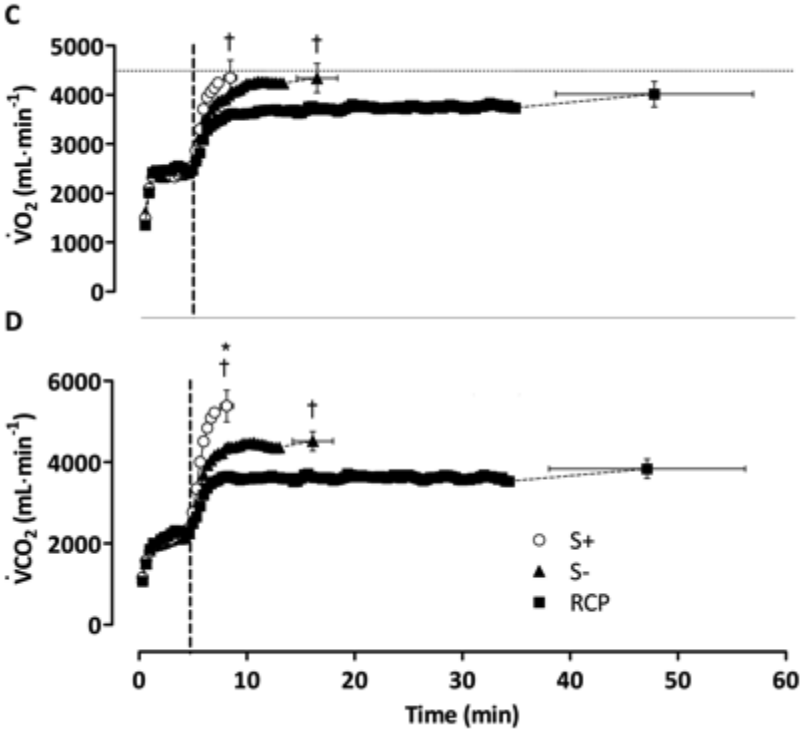
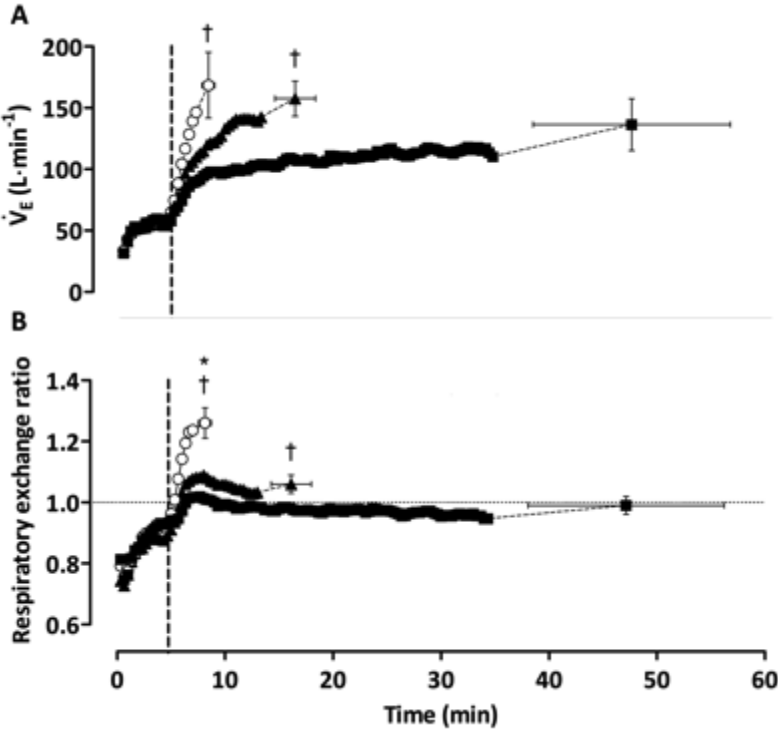
constant-load cycling exercise. Values are mean \pm SD.

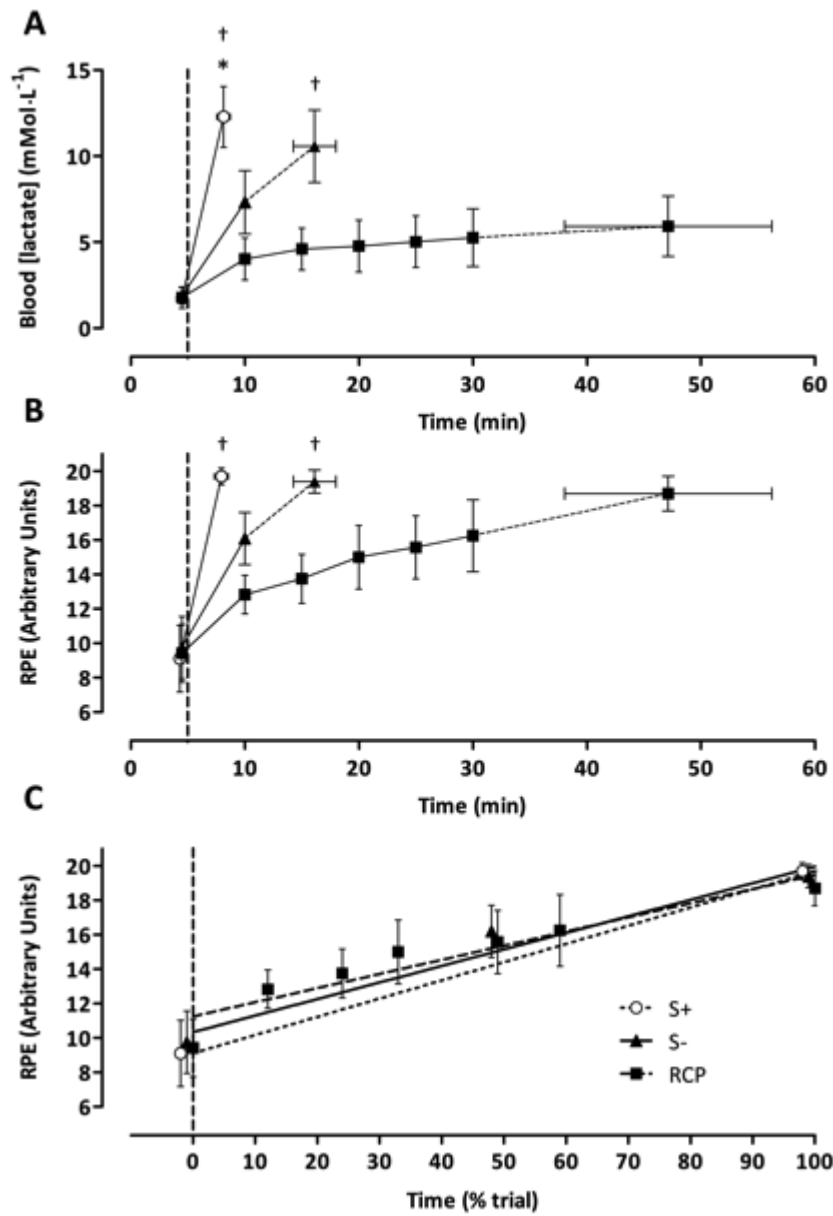
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Table 1. Motor evoked potentials (MEP), muscle compound action potentials (M-waves) and corticospinal excitability (MEP:M-wave) measured in vastus lateralis (VL) and rectus femoris (RF) during a sub-maximal contraction (20% MVC) pre- and post- severe- (S+ and S-) and heavy-intensity (RCP) constant-load cycling exercise. Values are mean \pm SD (n = 11).

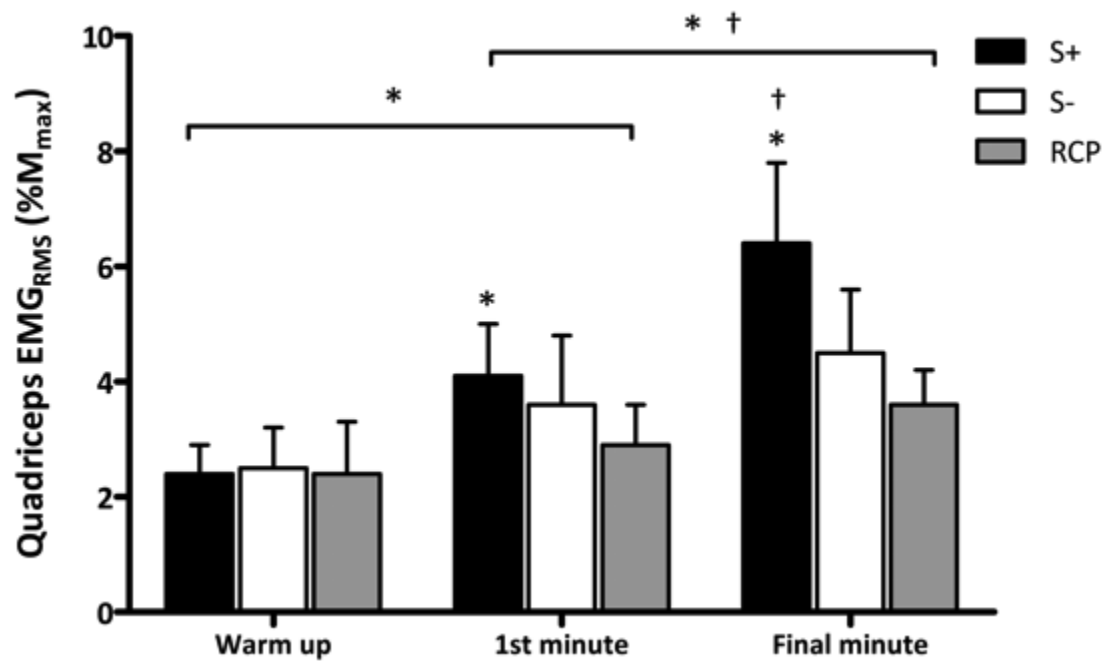
		S+	S-	RCP
<i>Rectus Femoris</i>				
MEP	Pre	2.95 \pm 2.20	3.11 \pm 1.95	2.43 \pm 1.46
amplitude (mV)	Post	2.64 \pm 1.28	2.42 \pm 1.58	2.34 \pm 1.84
M-wave	Pre	5.36 \pm 2.95	5.58 \pm 2.60	5.51 \pm 2.34
amplitude (mV)	Post	5.10 \pm 3.04	5.77 \pm 3.14	4.76 \pm 3.61
MEP / M-wave	Pre	52 \pm 16	54 \pm 18	44 \pm 18
(%)	Post	50 \pm 20	43 \pm 14	40 \pm 14
<i>Vastus Lateralis</i>				
MEP	Pre	3.46 \pm 2.15	2.26 \pm 1.35	2.88 \pm 2.29
amplitude (mV)	Post	2.94 \pm 1.33	1.89 \pm 0.76	1.74 \pm 0.83
M-wave	Pre	9.19 \pm 3.14	7.28 \pm 2.70	8.92 \pm 4.24
amplitude (mV)	Post	8.58 \pm 3.36	7.03 \pm 2.95	7.62 \pm 3.72
MEP / M-wave	Pre	36 \pm 16	31 \pm 11	33 \pm 17
(%)	Post	37 \pm 19	28 \pm 8	28 \pm 13





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